

WHAT IS CLAIMED IS:

8-13-2  
1. A method for identifying an essential chromosomal gene in a haploid test organism, said method comprising:

constructing a BAC-carrying merodiploid test cell by transforming a wild-type haploid host cell with a bacterial artificial chromosome (BAC) carrying a segment of DNA of the haploid test organism, which segment is homologous to a known segment of chromosomal DNA in the host cell, and wherein replication of the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the host cell;

inserting randomly a bacterial transposon into the merodiploid test cell so as to disrupt function of a gene therein;

culturing one or more of the BAC-carrying merodiploid test cells in a suitable culture medium while introducing the environmental condition so as to transform the merodiploid test cells into haploid test cells; and

identifying one or more of the haploid test cells that contain transposon-mutagenized DNA in an essential chromosomal gene therein.

2. The method of claim 1 further comprising obtaining the essential chromosomal gene by homology with a gene in the known segment of DNA.

3. The method of claim 1 wherein the identifying involves selection of test cells that do not survive subjection to the environmental condition as having the transposon in an essential chromosomal gene therein.

4. The method of claim 1, wherein the transposon is Tn5 or Tn10.

5. The method of claim 4, wherein the transposon is operatively linked to a first antibiotic resistance gene.

6. The method of claim 5, wherein the BAC comprises a second antibiotic resistance gene, wherein the first and second antibiotic resistance genes convey resistance to two different antibiotic compounds.

8. The method of claim 7, wherein the first and second antibiotic resistance gene provide resistance, respectively, to kanamycin and chloramphenicol.

10. The method of claim 1, wherein the BAC is temperature sensitive for replication and the environmental condition is a temperature that is selectively non-permissive for replication of the BAC in the test cell.

12. The method of claim 1, wherein the host cell is selected from the group consisting of *E. coli*, *Salmonellae*, and *B. subtilis*.

14. The method of claim 1, wherein the identified essential chromosomal gene has 100% sequence identity with a gene in the known segment of DNA.

15. The method of claim 1, wherein the identified essential chromosomal gene has at least 90% sequence identity with a gene in the known segment of DNA.

16. The method of claim 1, wherein the identified essential chromosomal gene has at least 80% sequence identity with a gene in the known segment of DNA.

17. The method of claim 1, wherein the BAC contains up to 100 genes of the haploid test organism.

18. The method of claim 1, wherein the haploid test organism and the host cell are the same species of prokaryote.

19. The method of claim 1, wherein a library of the BAC-carrying merodiploid test cells is constructed such that the BACs in the library collectively contain the entire genome of the haploid test organism.

20. The method of claim 19, wherein the entire genome of the haploid organism is contained in about 50 to 100 merodiploid test cells that each contain a unique segment of the genome of the haploid test organism.

21. The method of claim 19, wherein the test cells in the library are simultaneously subjected to the environmental condition.

22. The method of claim 1, wherein sufficient of the merodiploid test cells are constructed to provide four-fold coverage of the entire genome of the haploid organism.

23. The method of claim 1, wherein the BAC in the merodiploid test cell is contained within a fosmid/cosmid.

24. The method of claim 23, wherein the fosmid/cosmid is packaged in lambda phage prior to insertion into the host cell.

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25. A method for screening bacterial genes in a pathogenic bacterium whose genome is known to select compounds with putative antibiotic activity, said method comprising:  
constructing a BAC-carrying merodiploid test cell by transforming a wild-type haploid host cell with a BAC that carries a known segment of DNA of a pathogenic bacterium, which segment is homologous to a segment of chromosomal DNA in the host cell, and wherein the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the test cell;

inserting randomly a transposon into the merodiploid test cell so as to disrupt function of a gene therein;

culturing one or more of the merodiploid test cells in a suitable culture medium while introducing the environmental condition;

identifying one or more test cells that do not survive subjection to the environmental condition as containing the transposon in an essential chromosomal gene therein;

obtaining a corresponding essential gene in the known segment of DNA of the pathogenic bacterium by homology with the identified essential chromosomal gene in the test cell; and

screening the corresponding essential gene obtained from the pathogenic bacterium or a bacterial protein encoded by the corresponding essential gene against putative antibiotic compounds to determine those compounds that bind to or interrupt function of the corresponding essential gene or the bacterial protein, wherein such a compound is a candidate antibiotic against the pathogenic bacterium.

26. The method of claim 25, wherein the transposon is Tn5 or Tn10.

27. The method of claim 25, wherein the transposon is operatively linked to a first antibiotic resistance gene.

28. The method of claim 27, wherein the BAC comprises a second antibiotic resistance gene.

29. The method of claim 28, wherein the first and second antibiotic resistance genes are selected to provide resistance to the group consisting of ampicillin, tetracycline, kanamycin, and chloramphenicol.

30. The method of claim 29, wherein the first and second antibiotic resistance gene provide resistance, respectively, to kanamycin and chloramphenicol.

31. The method of claim 25, wherein the identifying includes subjecting the test cells to the antibiotics to which the first and second antibiotic resistance genes provide resistance.

32. The method of claim 25, wherein the BAC is temperature sensitive and the environmental condition is a non-permissive temperature for replication of the BAC in the test cells.

33. The method of claim 25, wherein the BAC is suppressor sensitive and the environmental condition is a suppressor that selectively prevents replication of the BAC in the test cells.

34. The method of claim 25, wherein the host cell is selected from the group consisting of *E. coli*, *Salmonellae*, and *B. subtilis*.

35. The method of claim 34, wherein the host cell is *E. coli*.

36. The method of claim 25, wherein the BAC contains up to 100 genes of the pathogenic bacterium.

37. The method of claim 25, wherein a library of the BAC-carrying merodiploid test cells is prepared such that the BACs in the library collectively contain the entire genome of the pathogenic bacterium.

38. The method of claim 37, wherein the entire genome is contained in about 50 to 100 merodiploid test cells that each contain a unique segment of the genome of the pathogenic bacterium.

39. The method of claim 38, wherein the test cells in the library are simultaneously subjected to the environmental condition.

40. The method of claim 25, wherein the candidate antibiotic is bactericidal.

41. The method of claim 25, wherein the bacterium is pathogenic in at least one mammalian species.

42. The method of claim 25, wherein the bacterium is pathogenic in at least one plant species.

43. A method for identifying an essential chromosomal gene in a haploid test organism, said method comprising:

constructing a BAC carrying a known segment of DNA of the haploid test organism, which segment is homologous to a known segment of chromosomal DNA in a haploid host cell;

inserting randomly a bacterial transposon into the BAC so as to disrupt function of a gene in the segment of chromosomal DNA;

introducing the BAC into the haploid test cell to create a merodiploid test cell;

culturing the merodiploid test cell in a suitable culture medium;

identifying one or more BAC-carrying merodiploid test cells that do not survive in culture as containing the transposon in an essential chromosomal gene therein; and

obtaining the identity of the essential chromosomal gene by homology with the known segment of DNA inserted into the BAC that was introduced into the identified test cell.

44. The method of claim 43, wherein the transposon is Tn5 or Tn10.

45. The method of claim 43, wherein the transposon is operatively linked to a first antibiotic resistance gene.

46. The method of claim 43, wherein the transposon is inserted randomly into the BAC *in vitro* prior to introduction of the BAC into the host cell.

47. The method of claim 46, wherein the known segment of DNA is linearized prior to introduction into the host cell.

48. The method of claim 43, wherein the host cell is an *E. coli* RecBC-SbcBC quadruple mutant.

49. The method of claim 43, wherein the identifying includes subjecting the test cells to the antibiotic to which the antibiotic resistance gene provides resistance.

50. The method of claim 43, wherein the host cell is selected from the group consisting of *E. coli*, *Salmonellae*, and *B. subtilis*.

51. The method of claim 43, wherein the BAC contains up to 100 genes of the test organism.

52. The method of claim 43, wherein a library of the BAC-carrying merodiploid test cells is prepared such that the BACs in the library collectively contain the entire genome of the test organism.

53. The method of claim 52, wherein the entire genome is contained in about 50 to 100 merodiploid test cells that each contain a unique segment of the genome of the test organism.

54. The method of claim 43, wherein the test organism is a pathogenic bacterium.

55. The method of claim 54, wherein the method further comprises screening a corresponding essential gene obtained from the pathogenic bacterium or a bacterial protein encoded thereby against putative antibiotic compounds to determine those compounds that bind to or interrupt function of the corresponding essential gene or the bacterial protein, wherein such a compound is a candidate antibiotic against the pathogenic bacterium.

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